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SULPHURIC ACID TREATMENT TO INCREASE GERMINATION OF BLACK LOCUST SEED

By H. G. Meginnis, *silviculturist, Southern Forest Experiment Station, Forest Service*

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THE PROBLEM AND THE INDICATED SOLUTION

The extensive planting projects undertaken as a part of the large-scale conservation program to control soil erosion have enormously increased the demand for the seed of black locust (*Robinia pseudoacacia* L.) and have at the same time called attention to the economic losses resulting from the failure of much of this seed to germinate. For example, the Mississippi Forest Service reports 29 percent germination from samples of 1 ton of black locust seed sown in 1933 for a Civilian Conservation Corps erosion-control project. This is, if anything, rather above the average for reasonably fresh domestic seed.

A large percentage of any lot of black locust seed will usually prove to be sound and capable of germination, but because the seed is very hard-coated, like that of a number of other members of the same botanical family (Leguminosae), germination may be delayed for a year or more, or may occur so sporadically that many of the belated seedlings have to withstand climatic conditions that they are as yet too immature to resist successfully, and consequently produce a poor stand of seedlings. Reports from nurseries engaged in growing this species show that seedling yields are exceptionally small, traceable frequently to failure of the seed to germinate promptly. Replies to a questionnaire sent out by the Forest Service in 1935, comprising reports from 42 nurseries located throughout the United States, indicate that on an average not more than 8 percent of sown seed produce nursery seedlings.

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To remedy this condition, several treatments have been developed for softening the seed coat of black locust or rendering it more permeable to moisture. Of these, the most important are (1) scarification, (2) soaking the seed in hot water until a majority of them have swollen, and (3) steeping in concentrated sulphuric acid.

Scarification, or abrasion of the seed coat by various mechanical means, has been successful with many kinds of seed, and special machines have been designed for this purpose. Machine scarification should prove effective with black locust seed provided mechanical details are worked out for controlling the severity of the treatment and adjusting it to the requirements of individual lots of seed. Recently attempts have been made to scarify black locust seed in quantities, and favorable increases in germination after such treatment have been reported. The hot-water treatment has been moderately successful in early tests, with the one serious drawback that it leaves the seeds in a softened and swollen condition which not only necessitates immediate sowing, but also renders handling more difficult and mechanical sowing impossible.

No previous conclusive tests of sulphuric acid treatment have apparently been made. The experiments described here demonstrate that steeping black locust seed in concentrated sulphuric acid for varying periods, usually 60 minutes, produces good results. The seeds not only germinate with satisfactory promptness, but when thoroughly dried after treatment they may be kept for a considerable period without appreciable loss in germinating capacity.

Comparative tests indicate that germination of acid-treated seed is in general markedly superior to that of seed soaked in hot water, and that a combination of the acid and water treatments produces no better and in some cases less satisfactory results than those from the acid treatment alone. Other tests indicate that germination of acid-treated seed compares favorably with that of scarified seed. Tests under field conditions demonstrate that acid treatment greatly increases the yield of seedlings.

The hot-water and scarification tests here described add little to earlier investigations beyond further confirmation with regard to the particular species in question. The sulphuric acid tests yield usable data in an unexplored and at present highly important field of forest planting, and give satisfactory evidences of the effectiveness of this method.

EARLY EXPERIMENTS IN SEED TREATMENT

The numerous authorities who have commented on the hardness of the black locust seed coats have generally advocated the use of "hot" water as a softening treatment but have differed considerably as to specific temperatures or periods of soaking. Toumey and Korstian (*12*)[1] recommend soaking in water at a temperature of 190° to 195° F., using four to five times as much water as seed, and setting the vessel aside until most of the seeds have swollen. Mattoon (*6*) suggests putting the seed in water at 160° and letting them soak at cooler temperatures for about 20 hours. That the soaking treatments should not be too prolonged has been demonstrated in studies by Toumey and Durland (*11*), which indicate that overlong soaking in water, particularly lukewarm or hot water, considerably reduces viability of tree seed.

[1] Italic figures in parentheses refer to Literature Cited, p. 34.

Various machines, differing widely in design and construction, have been developed by agronomists and agricultural engineers for scarifying hard seed of various farm crops. These machines usually operate on the principle of bringing seed forcibly in contact with an abrasive surface with sufficient impact to scratch or superficially rupture the coats.[2] Hurst et al. (*3*) describe recently developed barrel and disk scarifying machines specially designed for the treatment of legume seed.

During the past several years these and other scarifiers—particularly those of the barrel type—have been used at a number of nurseries to pretreat black locust seed, and although few if any published data are available to indicate whether the treatment increases yields of nursery stock, substantial increases in preliminary germination have been reported in some instances. Tumbling the seed for varying periods in an ordinary concrete mixer has been done at some nurseries to crudely scarify black locust seed. Chapman (*2*), after trials with various machines, reports failure of barrel scarifiers to effectively scarify some lots of hard-coated seed and difficulty in adjusting other available types of scarifying machines to eliminate undue breakage and injury during treatment. To overcome these defects, Chapman recommends use of the Ames scarifying machine developed in 1913 at the Iowa State College and more recently modified by him especially for treating black locust seed.

The Lake States Forest Experiment Station recommends the Denbigh scarifier,[3] patterned after a scarifying machine developed at the Bessey Nursery at Halsey, Nebr., and especially designed for thick-coated seed of the genus *Juniperus* and for other types of seed requiring a similarly severe treatment. Fisher et al.[4] report marked increases in germination of black locust seed after a 2-hour treatment in the Denbigh scarifier.

Treatment with sulphuric acid as a means of rendering hard seed coats more permeable has been recommended for many agricultural seeds, particularly those of clovers and other legumes, by Rostrup (*7*), Todaro (*10*), Love and Leighty (*5*), and many other early investigators. Show (*8*) is one of several who have recommended the sulphuric acid treatment for tree seed, having found that a soaking of 1 hour in 0.5- to 4-percent solution of sulphuric acid was effective with sugar pine seed. Larsen (*4*) found that germination of western white pine seed was increased by a soaking of 45 minutes in concentrated sulphuric acid with thorough rinsing afterwards. Spaeth (*9*) obtained better germination of basswood seed by partially digesting the outer pericarp with concentrated nitric acid followed by immersion in concentrated sulphuric acid for 12 to 30 minutes.

In view of the fact that sulphuric acid has proved a very effective agent for treating other leguminous seed, it is rather surprising that the method has not been thoroughly tested as a means of increasing and speeding up germination of black locust. The writer knows of but one earlier investigator who has reported on such tests.[5] Burton (*1*)

[2] Chapman (*2*) points out that impermeability of black locust seed is caused by a suberized and cutinized condition of the outer palisade cell walls rather than an impervious surface cuticle, hence he concludes that abrasion must extend through the cuticle and affect the walls of the underlying palisade cells in order to decrease impermeability.

[3] Described and illustrated in the Forest Research Digest, Lake States Forest Experiment Station, December 1935, pp. 4–5. [Multigraphed.]

[4] Fisher, P. L., Briggs, A. H., and Shirley, H. L. Unpublished data, Lake States Forest Experiment Station, 1936.

[5] The writer reported preliminary results and instructions for treating black locust seed with sulphuric acid in a mimeographed release dated May 9, 1934. Revised instructions were reported in the Southern Forest Experiment Station's Occasional Paper 47, issued in June 1935.

soaked black locust seed in concentrated sulphuric acid for 30 minutes with negative results. He attributed his lack of success to too long immersion in the acid.

PRELIMINARY TESTS IN THE TREATMENT OF BLACK LOCUST SEED

In order to determine to what degree certain lots of black locust seed are affected by hot-water treatment, 15 lots of seed from as many parent trees were collected during late July and early August 1933. Twelve lots were obtained near Holly Springs, Marshall County, in northern Mississippi, while the other three were collected near Natchez, Adams County, in the southwestern part of the State. The parent trees, mostly plantation grown, ranged in age from 3 to 23 years.

Samples of 100 seeds each were selected at random from each of the 15 lots and were germinated at ordinary room temperature in rolls of moistened cotton cloth for a period of 21 days, as described later for the final tests. Before the samples were put in the germinators, they were soaked for 23 hours in water which had an initial temperature of 170° F. Certain lots of seed failed to swell as a result of this treatment, while others responded readily. Germination for the individual lots in 21 days ranged from 7 to 64 percent.

Since there was a very apparent correlation between germination and initial swelling as a result of hot-water treatment, a number of soaking tests were made with all lots of seed, duplicating the water treatment which preceded the germination tests. These tests revealed that the three lots germinating best in the previous tests also showed the highest percentage of swollen seed after one hot-water treatment (67, 66, and 44 percent, respectively), while the two lots germinating least were almost unaffected by the treatment, only 2 and 13 percent, respectively, of the seeds swelling. So hard-coated were the latter two lots of seed that only 32 percent of one and 42 percent of the other had swollen after eight consecutive hot-water treatments comprising a total period of soaking amounting to 213 hours. In contrast, three treatments caused more than 90 percent of the two softest coated lots to swell.

Preliminary tests were next made to find, if possible, treatments which would induce the lots of harder coated seed to absorb water and germinate more completely. In general, the results of these tests indicated that (1) soaking in strong solutions of potassium hydroxide had no effect, (2) soaking in water was effective if the water had an initial temperature higher than 170° F., (3) steeping the seed in concentrated sulphuric acid was very effective provided the period of immersion exceeded 30 minutes.

The discussion to follow deals with additional tests, made in part for the purpose of checking the preliminary findings but more specifically to determine (1) the comparative effectiveness of sulphuric acid, hot water, and scarifying treatments; (2) the results of acid treatment measured in terms of seedling yield of black locust grown under field conditions; (3) the more important factors affecting acid treatment; and (4) the technique of treating large quantities of seed by this method.

COMPARATIVE TESTS WITH HOT WATER AND SULPHURIC ACID

METHODS

Seven lots of seed were used for these tests, of which three were bulk lots while four were from individual trees. The seven lots consisted of (1) the two softest coated lots (hereafter referred to as lots 1 and 2) of the 15 used in the preliminary tests; (2) the two hardest coated of the original 15 lots (lots 14 and 15); (3) a lot of Italian seed (lot A) 2 or more years of age; and (4) two lots (lots B and C) of fresh bulk seed collected locally in August 1933 from a number of trees near Holly Springs, Miss.

Samples of 50 seeds each were used in tests of lots 1, 2, 14, and 15, and of 200 seeds for the bulk lots A, B, and C. Only apparently sound seeds were used, but these were selected at random from thoroughly mixed samples without regard to size, color, or other characteristics.

Water treatment consisted of pouring water at temperatures of 65°, 170°, 190°, and 212° F. over the samples in open glass jars and letting the seed soak at room temperatures for 24 hours.[6] Fifty cubic centimeters of water was used to treat the larger samples, and 25 cubic centimeters to treat the smaller.

Similar samples were steeped at room temperatures in concentrated sulphuric acid (specific gravity 1.84), 95 percent pure, for periods of 30, 60, 90, and 120 minutes, using approximately two parts of acid to one part of seed. The seeds were stirred slightly with a stirring rod at the beginning of the test and again at the end. After treatment, the seeds were poured into containers made of 16-mesh screen and placed in cool, running tap water for 5 minutes to remove all acid.

Treated seeds were placed immediately in so-called "rag dolls" to germinate. These consist of cheesecloth strips, each 18 by 30 inches, folded in four thicknesses. Each lot of seed was placed in a marked compartment and the cloth strip rolled into a loose bundle. These bundles were soaked daily for 5 minutes in water at 90° F. and then drained of all free water. The bundles proved to be excellent germinators since they held moisture well and provided enough aeration to prevent the formation of molds in all but a few tests. They were kept in an ordinary living room in which temperature throughout the germination period varied from 65° to 85° F. during the day and dropped to a minimum of 46° to 55° at night.

Germination counts were made at 5-day intervals. Tests were terminated on the fifteenth day, since in the previous germination tests using this same method germination dropped off appreciably after the fifteenth day on an average. Furthermore, it was thought advisable to carry on the tests over a relatively short period, since the results would more closely approximate those obtained under more adverse field conditions.

RESULTS

As shown in table 1, the germination percentage was increased in all but two instances by treatment with hot water, but, except in one case, was either little affected or was somewhat lowered by soaking in water at a temperature of 65° F. It will also be noted that in every instance water heated to either 190° or 212° gave better results than did water at 170°. In 3 of the 13 separate tests, boiling water

[6] Tests showed that the temperature of boiling water dropped to that of the room (70°) in about 90 minutes.

gave results better by 13, 24, and 26 percent than did that 22° under boiling; in 5 tests boiling water excelled by 8 percent or less; in 2 tests the results were identical; and in the remaining 3 tests—lot 1, test 2, and lot A—the advantage was with the lower temperature. These data, although not conclusive, indicate that boiling water gives definitely better results with certain lots of seed, but with certain others may be less effective or barely more effective than water at a temperature of 190°. In general, the results confirm the usual recommendations that water slightly below the boiling point should be used in such treatments. Doubtless the quantity of water used is an influential factor in treatment by the hot water method, since this variant would largely determine whether initial temperatures are maintained for any length of time after the water is poured over the seed.

TABLE 1.—*Germination of black locust seed on fifteenth day, after soaking in water for 24 hours at different initial temperatures*

Seed lot and test	Check	At 65° F.	At 170° F.	At 190° F.	At 212° F.	Seed lot and test	Check	At 65° F.	At 170° F.	At 190° F.	At 212° F.
	Percent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*		*Percent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
Lot 1:						Lot A:					
Test 1	24.0	------	52.0	68.0	68.0	Test 1	72.0	68.5	74.5	77.5	64.0
Test 2	22.0	22.0	62.0	74.0	54.0	Test 2	68.5	62.0	74.5	78.5	77.5
Lot 2: Test 1	38.0	58.0	92.0	92.0	98.0	Lot B:					
Lot 14:						Test 1	12.0	6.0	18.5	28.5	41.5
Test 1	4.0	------	14.0	14.0	16.0	Test 2	15.0	12.5	29.5	46.5	70.5
Test 2	10.0	4.0	10.0	26.0	52.0	Lot C:					
Lot 15:						Test 1	31.5	29.0	32.0	44.0	49.5
Test 1	2.0	------	8.0	28.0	28.0	Test 2	39.0	29.5	44.5	66.5	74.5
Test 2	4.0	4.0	10.0	34.0	38.0						

The results of treating the seed with concentrated sulphuric acid are summarized in table 2. In every case germination was increased, and in all but three instances this increase was accomplished regardless of the period of immersion. The striking increase in all lots of seed except lot A was most marked in the case of the hardest coated seed, lots 14, 15, and B. Thus, in two tests only 2 and 4 percent of the checks from lot 15 germinated, as compared with "bests" of 94 and 98 percent under acid treatment. In lot B only 12 and 15 percent of the checks germinated, in contrast with 88 and 91 percent of the samples steeped in acid for 2 hours.

TABLE 2.—*Germination of black locust seed on fifteenth day after steeping in concentrated sulphuric acid for varying periods*

Seed lot and test	Check	Steeped for—				Seed lot and test	Check	Steeped for—			
		30 minutes	60 minutes	90 minutes	120 minutes			30 minutes	60 minutes	90 minutes	120 minutes
		Per-cent	*Per-cent*	*Per-cent*	*Per-cent*			*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
Lot 1:						Lot A:					
Test 1	24.0	62.0	50.0	48.0	46.0	Test 1	72.0	72.5	74.5	44.5	17.0
Test 2	22.0	36.0	74.0	60.0	60.0	Test 2	68.5	73.5	82.0	71.5	66.5
Lot 2: Test 1	38.0	88.0	96.0	90.0	90.0	Lot B:					
Lot 14:						Test 1	12.0	28.5	60.5	85.0	87.5
Test 1	4.0	62.0	92.0	80.0	78.0	Test 2	15.0	32.5	81.5	87.5	91.0
Test 2	10.0	66.0	82.0	72.0	48.0	Lot C:					
Lot 15:						Test 1	31.5	66.5	71.5	82.5	90.0
Test 1	2.0	66.0	70.0	92.0	94.0	Test 2	39.0	38.5	70.0	78.0	84.0
Test 2	4.0	54.0	82.0	98.0	90.0	Test 3	36.0	49.0	70.5	84.0	90.0

The best results from acid treatment were obtained by 60-minute steeping in 6 of the 14 separate tests—test 2 of lot 1, and lots 2, 14, and A. In six other tests—lot B, lot C, and test 1 of lot 15—the 2-hour treatment proved superior. In only two instances did either the 30-minute steeping or that of 1½ hours prove best. Lot 1, test 1, which germinated best after 30 minutes' treatment, gave still better results in a preliminary test treatment of 45 minutes. Lot A, of seed 2 or more years old, was least affected by acid treatment and was the only lot where too long treatment with acid apparently decreased germination below that obtained from untreated seed.

Since the water and acid tests were carried on simultaneously, comparisons may be made of the relative effectiveness of the best treatments of both (table 3). These data show that in all but four tests, acid treatment was more effective. Lots 1 and 2 germinated slightly better as a result of hot-water treatment, and acid and water treatments were approximately equal for lot A. Acid treatment was greatly superior for the other four lots. This is best illustrated by lot 15, in which the averages of both tests show that maximum germination percent was nearly three times as great for acid-treated seed.

TABLE 3.—*Comparative germination of black locust seed on fifteenth day following most favorable treatments with hot water and with concentrated sulphuric acid*

Seed lot and test	Check	Hot water	Sulphuric acid	Seed lot and test	Check	Hot water	Sulphuric acid
Lot 1:	*Percent*	*Percent*	*Percent*	Lot A:	*Percent*	*Percent*	*Percent*
Test 1	24	68	62	Test 1	72	78	74
Test 2	22	74	74	Test 2	68	78	82
Lot 2: Test 1	38	98	96	Lot B:			
Lot 14:				Test 1	12	42	88
Test 1	4	16	92	Test 2	15	70	91
Test 2	10	52	82	Lot C:			
Lot 15:				Test 1	32	50	90
Test 1	2	28	94	Test 2	39	74	84
Test 2	4	38	98	Test 3	36	----	90

In order to check these data further, using a different germinating medium, 200 seed samples each of lots 15 and B were given optimum water and acid treatments as indicated in tables 1 and 2 and sown thickly in drills in sand flats. In connection with these tests, some of the samples were subjected to a combined extreme acid and water treatment, i. e., the seeds were soaked in sulphuric acid for 2 hours, then boiling water was poured over them, and they were allowed to soak in this at room temperatures for 24 hours. The sand flats were subjected to the same room temperatures as were used in the previous germination tests and were watered daily. Germination counts were made at 5-day intervals for a period of 45 days.

The results are indicated in table 4 and figure 1, the former summarizing the germination of both lots of seed and the latter the percentage of germination for lot 15. It will be noted that germination proceeded at a slower rate than when the rag dolls were used, but the comparative results are similar and show superior germination from seed treated with sulphuric acid. As shown in figure 1, only 13 percent of the untreated seeds from lot 15 germinated as compared with 52 percent of the water-treated seeds and 73 percent of those treated with acid. With both lots, acid or water treatment alone gave somewhat better results than did the combined treat-

ment.[7] The appearance of the seedlings at the end of 21 days is shown in figure 2.

The foregoing results indicate that sulphuric acid treatment is an effective and practical means of stimulating and increasing germina-

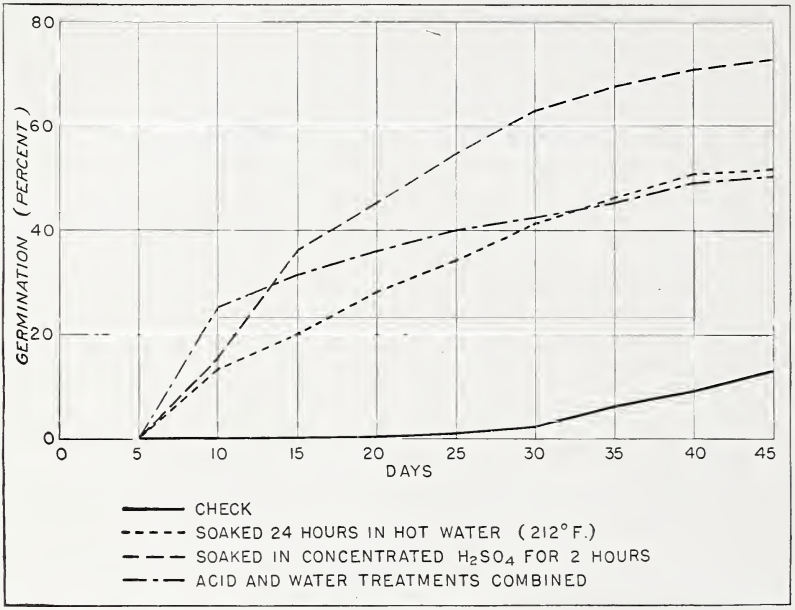

FIGURE 1.—Germination percentages at 5-day intervals of seeds from lot 15 submitted to water, acid, and acid-water treatments.

tion of black locust seed, especially with lots of harder coated seed. Acid-treated seed remain hard and their shape and size are unchanged, whereas the soft, swollen seed resulting from the hot-water treatment must be handled more carefully, and resulting irregularities in their

TABLE 4.—*Cumulative germination (number of seedlings) at 5-day intervals of treated and untreated black locust seed in sand flats* [1]

Lot and seed treatment	5 days	10 days	15 days	20 days	25 days	30 days	35 days	40 days	45 days
Lot 15:									
Check—no treatment	0	0	1	1	2	5	13	18	26
Soaking in hot water	0	27	41	57	69	82	92	101	104
Steeping in acid	0	30	72	90	109	126	135	142	146
Acid and hot water	0	50	64	73	80	85	91	98	102
Lot B:									
Check—no treatment	0	2	20	40	56	65	69	75	77
Soaking in hot water	1	90	101	110	118	120	123	125	125
Steeping in acid	0	55	102	125	138	154	165	171	177
Acid and hot water	2	42	73	86	96	95	96	96	93

[1] 200 seeds per sample.

size and shape make planting in mechanical seeders impossible. Another serious disadvantage of the hot-water treatment is that the seed must be sown at once to prevent deterioration and lowered viability. These disadvantages of hot-water treatment apply to a much greater degree when large lots of seed must be treated and sown.

[7] Combined acid and water treatment was also tested in conjunction with the previous tests (tables 1 and 2) using all lots of seed. The treatment either decreased germination of certain lots or gave results no better than were obtained by acid treatment alone.

TESTS WITH SCARIFIED AND ACID-TREATED SEED

During August 1934 some field tests were made at a large black locust nursery operated by the Mississippi Forest Service near Holly Springs to compare germination of seed treated with sulphuric acid and samples of the same lot scarified in three ways. These tests were run as an adjunct to other experiments undertaken at the suggestion of Carl Hartley, principal pathologist of the Bureau of Plant Industry, to check the relation of several modes of pretreatment to fungus attacks on seedlings during the period of germination and emergence from the soil. Earlier in the season heavy losses attributed to soil organisms of the damping-off group had occurred in this nursery.

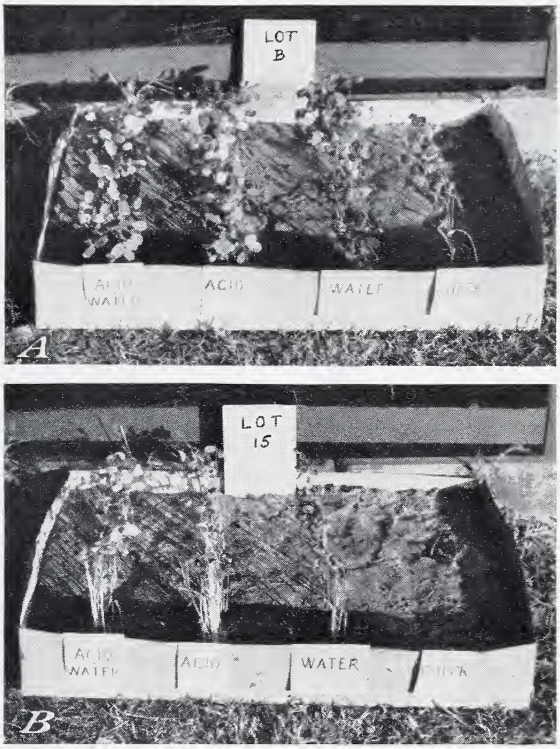

FIGURE 2.—Appearance of seedlings germinated in 21 days from two lots of treated and untreated black locust seed: *A*, lot B; *B*, lot 15.

A working sample of seed, representing the bulk lot sown in the nursery earlier in the season, was divided into fifths; one portion was left untreated, while a second was steeped in sulphuric acid for 30 minutes at temperatures varying from 66° to 70° F., washed in running water for 5 minutes, and dried quickly under an electric fan. The remaining portions were scarified[8] in barrel and disk scarifying machines developed by the Bureau of Agricultural Engineering and described by Hurst and others (*3*). Treatments nos. 1 and 2 consisted of tumbling the seed for 10 and 20 minutes, respectively, in a barrel scarifier containing 5 pounds of ½- to ¾-inch gravel as the abrasive

[8] By representatives of the Bureau of Plant Industry at Statesville, N. C., and Washington, D. C.

agent, the barrel being rotated at a speed of 50 to 60 revolutions per minute, as recommended by the designers. Treatment no. 3 consisted of moderate scarification in the disk scarifier. In this machine, seed is scarified by subjecting it to friction developed by a carborundum-surfaced plate revolving against a stationary rubber disk.

On August 21, 500-seed samples of each treatment were sown thickly in drills three-fourths of an inch deep and spaced 17 inches apart to provide a series of 5 randomized blocks located on alluvial sand deposited by previous overflow waters of an adjacent creek. The same number of samples was sown similarly in five randomized blocks on a well-drained loam located in another part of the same nursery. Seedling counts were made 11, 15, and 21 days after sowing and the tests were completed on September 10. The beds were watered daily or every few days as needed. The period was one of humid, hot weather.

Analysis of the data reveals that variance due to treatments was highly significant on both loam and sand. Table 5 shows average germination percents by treatments and soils. Germination for all treatments was markedly less on loam than on sand, the differences being attributable to the decimating influence of fungi on the finer textured soil, as indicated by molding of seed during emergence and typical damping-off losses while seedlings were in the cotyledon stage. Acid-treated seed germinated best on both loam and sand followed, in order, by scarified seed of treatments nos. 1, 2, and 3. On loam, mean differences between acid-treated and barrel-scarified seed are slight and within the limits of experimental error; each of these treatments, however, produced significantly better germination than did scarification treatment no. 3. The latter gave no significant increase in germination over the checks, and seed so treated were observed to be especially vulnerable to fungus attacks. On sand, where fungus damage was of no consequence, the acid treatment and all three methods of scarification significantly increased germination, the average increase being more than twofold.

TABLE 5.—*Mean germination percents* [1] *in 21 days of black locust seed treated with sulphuric acid and scarified by three methods* [2]

Soil	Check	Sulphuric acid treatment	Scarifying treatment no. 1	Scarifying treatment no. 2	Scarifying treatment no. 3	Standard error of difference [3]
Loam	17.3	31.8	31.2	29.0	18.3	3.1
Sand	24.6	57.1	55.1	53.7	51.1	4.3

[1] Averages based on a total of five 500-seed samples of each treatment sown in 5 randomized blocks on each type of soil.
[2] Scarified as follows: Treatment no. 1—rotated in barrel scarifier for 10 minutes; treatment no. 2—same treatment, doubled; treatment no. 3—moderate treatment in disk-type scarifier.
[3] Difference in mean germination considered significant if more than 2.5 times the standard error of the difference.

The data indicate that for this particular lot of seed, acid treatment or rotation for 10 minutes in a barrel scarifier gave optimum results. Doubling the latter treatment apparently resulted in slight overtreatment, although there were no visible evidences of greater injury to seed subjected to the longer period of scarification. Scarification in the disk machine was obviously too harsh treatment for optimum results judged not only by lowered germination but also by the fact that this treatment caused notably more breakage to seed than did the others, although

not more than 3 percent of the seed treated in this manner were so affected. It will be noted, however, that in spite of overtreatment, this mode of scarifying gave a highly satisfactory increase in germination over untreated seed on sand. Under the more adverse pathological conditions encountered on loam, however, losses attributable to overtreatment cancelled any such increases. Doubtless drastic pretreatment by any method increases the likelihood of disease and seed spoilage, particularly if the seed must remain for extended periods in contact with soil when lack of moisture, low temperatures, or other factors preclude prompt germination. For this reason it is important that pretreatments be properly controlled and applied judiciously. Overtreatment of seed is particularly to be avoided, inasmuch as it may nullify or at least greatly decrease effectiveness of pretreatment or, in exceptional cases, may actually produce a poorer stand of seedlings than no treatment at all.

Although these data indicate that acid treatment compares favorably with two well-known methods of scarification, no general conclusions can be drawn as to the relative merits of the two types of treatment. To do this would require comprehensive tests using a great variety of seed treated according to carefully established optima for each method. Both treatments, when judiciously applied, are effective methods of inducing greater germination of hard seed, and both methods have a common advantage over hot water treatment in that they leave the seed in sound condition for sowing. To be fully effective and safe, scarification and acid treatments alike require advance knowledge of the character of seed to be treated and adjustment of treatment details to meet these requirements. This entails, in the case of scarification, experimentation and control over a number of mechanical variables; and, with acid treatment, the regulation of temperatures and period of immersion in the chemical. Scarification provides a rapid, relatively cheap method of treating large quantities of seed. An advantage of acid treatment is that it requires only a supply of acid plus materials commonly at hand, which makes the method especially well adapted to treatment of small lots of seed, as well as of large quantities at nurseries where the character of the output may not warrant an investment in special machinery.

FIELD TESTS OF ACID TREATMENT

During the 1934 growing season additional tests were carried on in a nursery at Holly Springs to obtain a further check on the effectiveness of sulphuric acid treatment under less favorable germinating conditions, and particularly to determine the effect of the treatment on seedling yield of black locust.

METHODS

In these tests four lots of seed were used—lots B and C employed in previous tests and two other fresh bulk lots, D and E, collected in the locality during August 1933. Lots B and C were steeped in concentrated sulphuric acid for 120 minutes while lots D and E were similarly treated for 60 minutes. All four lots were treated on May 14 and stored dry for 31 days before they were sown.

The nursery soil, Memphis silty clay loam, was plowed and harrowed twice during the spring, and on the sowing date was in excellent condition. Four rows of treated seed, alternated with an equal

number of rows of untreated seed, a row of each representing each lot, were sown on June 14. The rows were 375 feet in length and were spaced 24 inches apart to permit easy cultivation. Seed was covered to a depth of one-half to three-fourths inch. Although the sowing rate varied, the quantity of seed sown in each row was accurately determined by weighing to the nearest 0.05 g. The row sowings were also supplemented by more detailed tests. In each of the above rows, four test sections were staked out at intervals of 66 feet. Each test section was 48 inches in length and in each were sown 100 sound seeds of the lot and treatment corresponding with that sown in the row. These tests, 16 in number, received the same cultural treatment as did the rows in which they were located, but in addition they were examined at 5- and 10-day intervals throughout the growing season, at which times seedling and mortality counts were made.

The seedlings were not watered artificially and were weeded and cultivated only three times during the season. Although rainfall for the months June to August, inclusive, totaled 9.43 inches, or only 79 percent of the normal amount,[9] growing conditions were on the whole satisfactory. Germination, however, was undoubtedly decreased by severe surface crusting of the soil following a rain of 3.02 inches on June 18. Drought, damping-off fungi, and extreme heat, especially during early July, were directly responsible for the death of many seedlings.

RESULTS

In figure 3 is shown the total number of living seedlings in all 16 tests combined, as tallied at 5- and 10-day intervals throughout the season. It will be noted that in the case of acid-treated seed the number of living seedlings reached a peak on June 29, 9 days earlier than did those from untreated seed. As is also indicated, 1,600 untreated seeds used in these tests produced a total crop, as tallied on September 16, of 122 seedlings, whereas the same number of acid-treated seeds produced 309 or 2.5 times as many seedlings.

Comparisons between individual tests reveal that in 14 of the 16 tests acid-treated seed excelled, yielding from 1.4 to 28 times as many seedlings as the checks. In the two remaining tests the checks produced 1.1 and 1.4 times as many seedlings as did acid-treated seed. The most outstanding differences were obtained in two tests with lots C and D, in which acid-treated seed produced 20 and 28 seedlings respectively while untreated seed produced only 2 and 1.

Comparative germination and yield of seedlings from treated and untreated seed of each of the four lots, as indicated by the average of four tests, are shown by diagram in figure 4. On an average, germination of acid-treated seed of lots B, C, D, and E was 2.1, 2.1, 8.1, and 2.7 times as great respectively as that of untreated seed. The average yields of seedlings from acid-treated seed were 1.7, 2.4, 14.6, and 2.1 times as great as from untreated seed. Combining the data for all lots of seed shows that on an average 19.3 percent of the acid-treated seed produced plantable nursery seedlings compared with a seedling yield of only 7.6 percent from untreated seed. This difference is too great to be ascribed to chance.

[9] Based on Forest Service rainfall data for June, July, and August and compared with the U. S. Weather Bureau 46-year average for the locality.

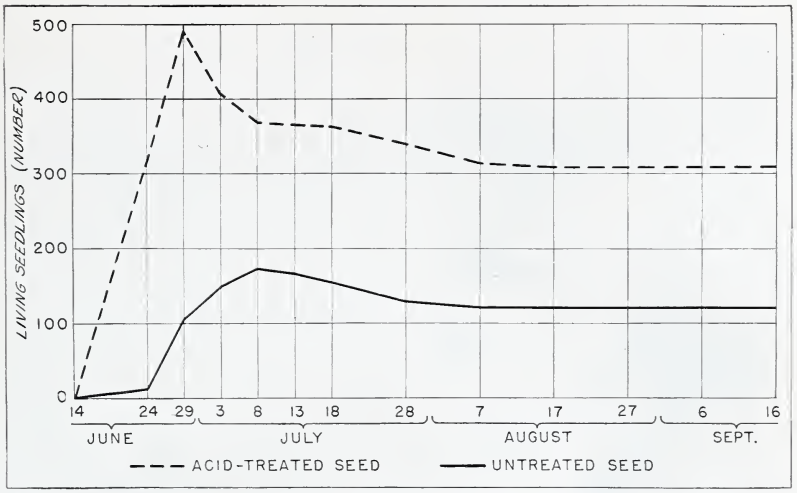

FIGURE 3.—Seedlings alive on periodic dates; grown from acid-treated and untreated black locust seed of four different lots and distributed in 16 field tests of 100 seeds each.

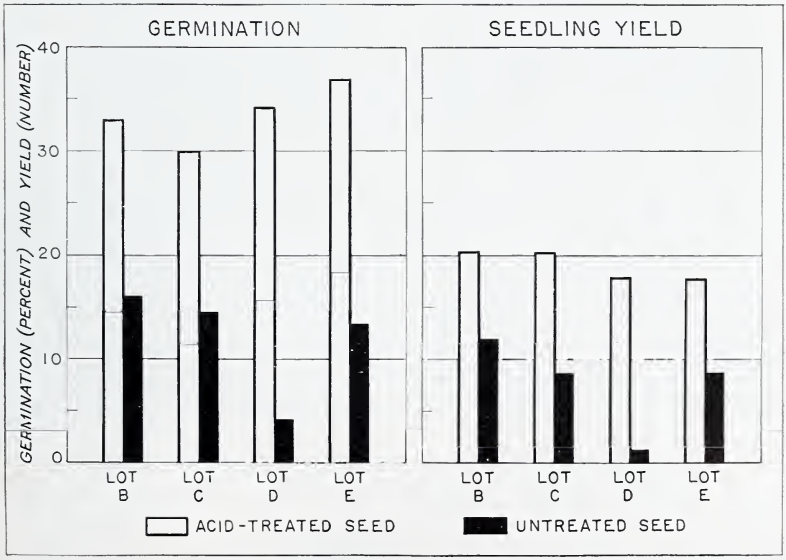

FIGURE 4.—Comparative germination and yield of seedlings from four lots of acid-treated and untreated black locust seed, based on averages from four field tests of 100 seeds each.

The yield of seedlings from the more extensive row plantings as tallied on September 27 are summarized in table 6. It will be noted that in every instance the rows sown to treated seed produced

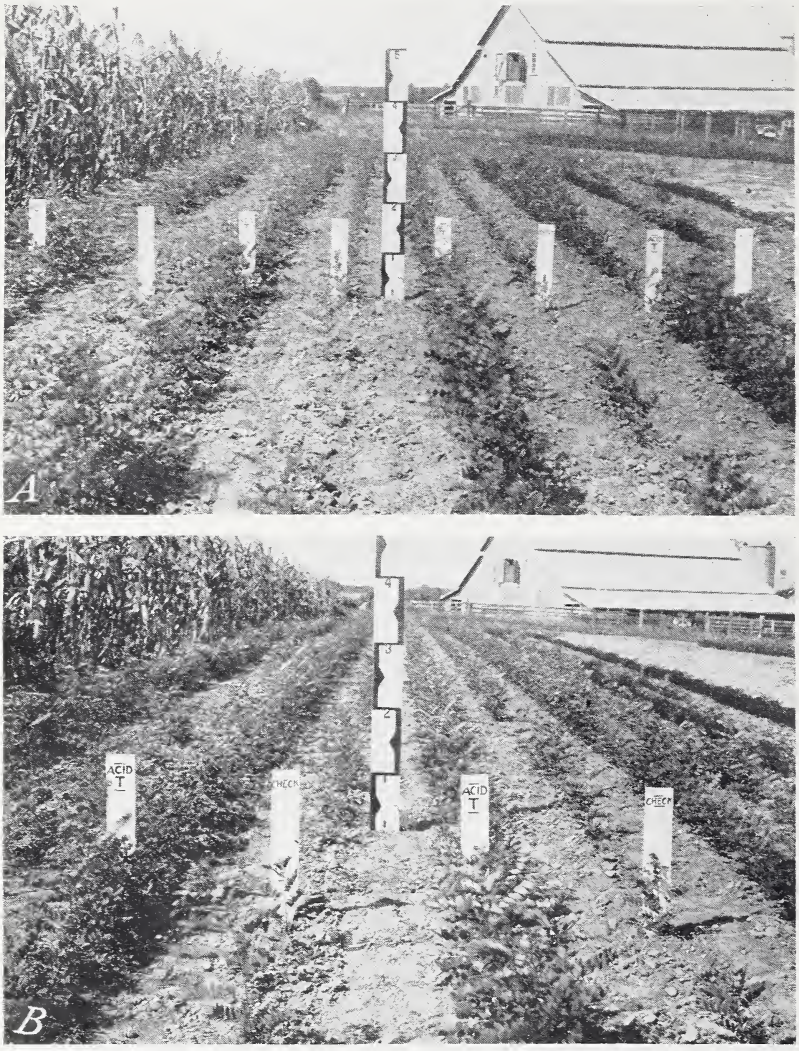

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FIGURE 5.—Comparative stand of black locust seedlings from acid-treated and untreated seed sown 56 days previous (scale in feet). *A*, Portion of all eight rows used in four tests; two rows each sown with seed of lots B, C, D, and E, in order named from left to right. *B*, More detailed view of seedling stand from treated and untreated seed of lot C (left) and lot D (right).

markedly superior stands of seedlings (fig. 5). The effectiveness of acid treatment is best expressed by comparing the number of seedlings produced by treated and untreated seed per pound of seed sown.

TABLE 6.—*Summary of seedlings produced by four lots of acid-treated and untreated black locust seed in nursery sowings, season of 1934, Holly Springs, Miss.*

Seed lot and treatment	Quantity of seed sown [1]		Seedlings in final crop	Seedlings per pound of seed [2]	Increase, acid-treated seed
Lot B:	*Pounds*	*Number*	*Number*	*Number*	*Percent*
Sulphuric acid	0.300	6,980	1,157	3,860	114.4
Check	.217	5,050	390	1,800	
Lot C:					
Sulphuric acid	.291	5,820	876	3,010	90.5
Check	.340	6,800	538	1,580	
Lot D:					
Sulphuric acid	.373	7,830	853	2,290	94.1
Check	.238	5,000	281	1,180	
Lot E:					
Sulphuric acid	.276	6,900	1,176	4,260	81.3
Check	.328	8,200	770	2,350	

[1] Approximate number of seed per pound as determined by weighings and counts: Lot B, 23,250; lot C, 20,000; lot D, 21,000; lot E, 25,000.
[2] Rounded to nearest 10.

TESTS OF FACTORS AFFECTING ACID TREATMENT

During the early part of the 1934 season, attempts to treat large quantities of seed for nursery sowing led to additional tests of several factors that were suspected of affecting results obtained from acid treatment. Questions which arose in this connection were: (1) Is temperature a factor of sufficient importance to warrant control during treatment? (2) Is prolonged washing of seed following treatment injurious? and (3) Does drying and subsequent storage of treated seed prior to sowing impair viability?

EFFECT OF TEMPERATURE

In most of the tests previously cited, air temperatures, although usually observed, were not controlled during the period acid and seed were in contact, and treatments in all instances were made at normal room temperatures varying from about 60° to 80° F. Within this range, variations in temperature produced no outstanding or noticeable effects, seed of a given lot usually responding similarly to treatment so long as the period of immersion in the chemical remained fairly constant. In treating seed after the advent of hot weather, however, it was discovered (somewhat accidentally) that the action of sulphuric acid is considerably intensified at higher temperatures and may damage seed unless proper allowance is made by reducing the period of treatment. This temperature effect was first noted when several small lots of seed were treated early in June according to a previously established optimum immersion period based on room temperatures, and were observed to be seriously injured by the later treatment at which time temperatures exceeded 95°. In another instance, 50 pounds of seed were completely charred by immersion in acid for 1 hour on a hot day with temperatures near 100°. With this particular lot of seed it had been conclusively demonstrated in earlier tests that a 1-hour treatment left seed in sound condition and was optimum under temperatures existing at that time, namely 70° to 80°.

These observations led to tests in which temperature was made the variant. The adverse results of treating seed at abnormally high temperatures as revealed in these experiments were so noticeable as to scarcely require confirmatory germination tests—a considerable percentage of the seed so treated being corroded by the chemical. Data in table 7 representing the results of some of these tests illustrate the effect on germination of treating samples of two lots of seed for 1 hour at 68° and 107° F., respectively, and the effect of cutting the immersion period in half when treating at the higher temperature.

TABLE 7.—*Comparative germination of black locust seed on fifteenth day* [1] *following treatment with sulphuric acid at normal room temperature (68° F.) and at a high temperature (107° F.)*

Seed lot [2] and test	Steeped in sulphuric acid 60 minutes at 68° F.	Steeped in sulphuric acid 60 minutes at 107° F.	Steeped in sulphuric acid 30 minutes at 107° F.
Lot 2:	*Percent*	*Percent*	*Percent*
Test 1	47.8	2.4	90.2
Test 2	60.8	13.2	93.8
Average [3]	54.3	7.8	92.0
Lot 15:			
Test 1	53.4	14.2	56.0
Test 2	64.2	24.4	59.8
Average [3]	58.8	19.3	57.9

[1] Tests made in sand germinating beds located outdoors; 500 seeds of each treatment sown in each test. Beds watered daily.
[2] Same lots used in earlier tests, lot 2 being soft-coated and lot 15 exceedingly hard-coated.
[3] Standard error of the difference between treatment means: Lot 2, 7.1; lot 15, 6.3.

Germination of both lots was markedly reduced by treatment at the higher temperature, particularly the soft-coated lot 2. On an average, only 8 percent of the seed of this lot germinated after treatment at 107° F. whereas treatment for the same period at 68° gave mean germination of 54 percent. With the hard-coated lot 15, the reduction in germination was less pronounced but nevertheless considerable, treatment at the lower temperature causing on an average 59 percent of the seed to germinate whereas germination of only 19 percent was obtained with seed treated at the higher temperature.

Furthermore, the data show that by immersing the seed in acid for 30 minutes instead of 1 hour when treating at a temperature of 107° F., germination was obtained which compares favorably with that resulting from 1 hour's treatment at 68°; in fact, the former combination gave an increase with lot 2 presumably attributable to the fact that 1 hour's treatment at 68° resulted in undertreatment whereas the combination of 30 minutes' treatment at 107° approached the optimum for this particular lot.

These results confirm observations previously cited and show conclusively that temperature must be controlled within approximate limits while seed remain in contact with acid. In applying this principle to seed treatment, it is not practicable to work out, experimentally, temperature-time schedules that will apply to all lots of seed owing to great variations in seed-coat structure among different lots. For optimum results the proper combination of time in acid and temperature must be determined in advance of actual treatment for each lot of seed. Experience indicates that the simplest, safest, and

most satisfactory system of treating any lot of seed with acid is as follows: (1) Determine the optimum period of immersion in acid in preliminary tests made when air temperatures range from 60° to 80° F.; and (2) carry on actual treatments when temperatures approximate (roughly ±5°) those in effect during the preliminary test period. Adherence to this system calls for additional preliminary tests if seed are to be treated when temperatures deviate from allowable limits. The optimum length of treatment may be worked out in preliminary tests for any given range of temperatures, including those abnormally high, and seed treated accordingly. There are obvious advantages, however, in limiting preliminary tests to times when temperatures fall within 60° to 80°, since the latter are normally in effect during most seasons or can be artificially duplicated with little difficulty; furthermore, the consequences of deviating slightly from allowable temperature limits are much less serious at normal room temperatures than at temperatures of 100° or over.

EFFECT OF PROLONGED WASHING

Treatment of seed with sulphuric acid entails washing afterward to remove the chemical. With small samples of seed, amounting to a few pounds or less, 2 to 5 minutes under a stream of tap water is usually ample to free the coats from harmful chemical; and with larger lots up to 50 pounds, washing for about 10 minutes will sufficiently cleanse the seed. There is therefore no particular necessity of keeping treated seed in contact with water for extended periods, but the fact that some washing is required gives rise to the question of whether it is possible to injure treated seed by overlong cleansing.

When seed have not been overtreated by too long steeping in the acid, they remain sound, unswollen, and with outer coats intact and unmarred by pitting or other evident signs of corrosion. In this condition they appear fully as resistant to soaking in cool water as do untreated seed, and hence should be exposed to no unusual hazards during this phase of the treatment, inasmuch as soaking in water at room temperatures for more extended periods does not usually induce premature swelling and germination of most lots of sound seed (table 1).

Numerous trials were made with many batches of treated seed washed for the usual periods after soaking in acid. Detailed scrutiny of such samples before and after washing failed to disclose physical injury to embryo or outer coats as a result of washing treatments. Exceptions were samples of seed intentionally immersed in acid for too long a period or unsound seed, including those shriveled, broken, or otherwise worthless which occur in relatively small amounts in practically all bulk lots. Seed of these types are frequently left by the acid in a softened or partially swollen condition and are visibly further damaged by contact with running water, particularly when the latter is applied under strong pressure and for unnecessarily long periods.

In order to check observations and probe the possibility of impairing germination of properly treated seed by excessive washing, some tests were made with seed of an especially soft-coated lot (lot 2). Data in table 8 compare germination in 13 days of untreated seed and seed given an optimum acid treatment and afterwards washed for 2½, 10, and 30 minutes, respectively. Mean germination percents

for these treatments show slight but in the main significant differences due to prolonged washing. The mean difference between seed washed for 2½ minutes and those washed for 30 minutes amounts to 4.1 percent, 93.4 percent of the seed washed for the shorter period germinating, compared with 89.3 percent of those washed unnecessarily long. This difference in mean germination, although small, is significant, amounting to about twice the standard error of the difference between means. The difference in mean germination for seed washed 2½ minutes and those washed 10 minutes amounts to only 2.4 percent, which is not considered significant.

TABLE 8.—*Germination in 13 days of a lot of soft-coated black locust seed steeped in sulphuric acid for 60 minutes at 70° F. and washed in tap water for varying periods to remove the acid*

Seed lot and test [1]	Check—no treatment	Steeped in sulphuric acid 60 minutes at 70° F., washed 2½ minutes	Steeped in sulphuric acid 60 minutes at 70° F., washed 10 minutes	Steeped in sulphuric acid 60 minutes at 70° F., washed 30 minutes
Lot 2:	*Percent*	*Percent*	*Percent*	*Percent*
Test 1	18.0	90.8	90.0	88.8
Test 2	19.0	96.0	92.0	89.8
Average [2]	18.5	93.4	91.0	89.3

[1] Tests made in sand bed located outdoors and watered daily; 500 seeds of each treatment sown in each test.
[2] Standard error of the difference, 2.0.

These data indicate that washing seed for unnecessarily long periods reduces germination and should be avoided. With seed soaked in acid for optimum periods, the indications are that prolonged washing will not prove seriously injurious. Seed, however, which remain too long in acid or have otherwise been given harsh treatment may suffer additional injury if washing exceeds the short periods required to free the coats from the adhering chemical.

EFFECT OF DRYING AND STORING SEED AFTER TREATMENT

To determine whether thorough drying and storage of black locust seed for a reasonable time after acid treatment results in loss in viability, duplicate tests were made with samples from lots B, C, and D. Samples of 200 seeds each were treated with acid for the optimum period and placed in germinating cloths, some immediately, some 24 hours later, and some after 7 days. The two latter groups of seed were spread out after treatment and allowed to dry thoroughly at room temperature.

The results (table 9) show no significant differences in germination between seed kept 24 hours or 7 days after treatment and those put to germinate immediately. These results, indeed, agree with those obtained by Love and Leighty (*5*), who found that the viability of red clover seed dried for 34 days after sulphuric acid treatment was unimpaired, such seed germinating as well as seed planted immediately. Cottonseed and seed of various legumes responded similarly after acid treatment and subsequent drying.

TABLE 9.—*Germination at the end of 15 days of samples of 200 acid-treated black locust seed* [1] *dried for different periods after treatment*

Seed lot and test	Check	Germinated immediately	Dried 24 hours	Dried 7 days
Lot B:	*Percent*	*Percent*	*Percent*	*Percent*
Test 1	8.5	86.5	91.0	85.5
Test 2	11.0	86.0	81.0	88.0
Average [2]	9.8	86.2	86.0	86.8
Lot C:				
Test 1	34.5	88.5	85.0	87.0
Test 2	22.0	83.5	84.5	83.0
Average [2]	28.2	86.0	84.8	85.0
Lot D:				
Test 1	13.5	86.0	([3])	84.0
Test 2	21.5	84.5	([3])	89.0
Average [2]	17.5	85.2		86.5

[1] Optimum treatment as indicated by earlier tests.
[2] Standard error of difference: Lot B, 3.8; lot C, 5.0; lot D, 3.9.
[3] No tests.

Later experience indicates that acid-treated seed can be kept for much longer periods without deteriorating appreciably in quality. Seed used in the 1934 field tests, previously cited, were treated 1 month prior to sowing with no apparent diminution in viability at the end of this period.

Over comparatively long periods of time, however, it is probable that treated seed deteriorate somewhat more rapidly than untreated seed. In a nursery test at Holly Springs in 1935, seed treated 1 year previously were compared with seed of the same lot which had been stored identically but were freshly treated with sulphuric acid (under closely duplicated temperature conditions) just prior to sowing. Since no early observations were made, the effect of treatments on initial germination cannot be compared, but a seedling tally at the end of the growing season disclosed that treatment 1 year prior to sowing produced on an average only 70 percent as many seedlings as did the fresh treatment.

Probably the rate and extent of deterioration of treated seed is conditioned by the original character of the outer coats, the severity of pretreatment, and factors of storage. When seed have been properly treated and stored in a dry condition, they apparently undergo no appreciable loss in viability during the first month or so, and such changes as occur later doubtless take place at a relatively slow rate and are of little practical consequence. While it is theoretically good practice to sow seed soon after treatment, there appear to be no hazards whatever involved in treating seed some weeks in advance of actual sowing.

EXPERIMENTAL APPLICATION OF RESULTS TO NURSERY-SCALE TREATMENTS

In applying these results to treating quantities of seed for nursery sowing, problems not encountered in small-scale experimental treatments present themselves. One of these arises from the great variability in hardness within a single lot of seed. Another is the difficulty

of maintaining proper control over temperatures during the treating period.

For these reasons, treatment of any specific lot or lots of seed requires preliminary tests (1) to indicate whether acid treatment is needed, and, if so, (2) to determine how long the seed should be soaked in acid for optimum results. Tests for either purpose necessitate reasonably careful, standardized procedure in sampling the bulk lot of seed to be treated and in conducting simple trial treatments.

SAMPLING AND PRELIMINARY GERMINATION TESTS

When a bulk lot of seed to be treated was known to include lots from different localities, or when the various groups of seed differed noticeably in size, color, or other characteristics, giving reason to suspect varying reaction to treatment, it was found preferable to segregate and sample each batch and conduct a series of independent tests to ascertain the treatment requirements of each. When the seed in all sacks appeared homogeneous, or if there was no reason to suspect differences in origin, the entire lot was treated as a unit in sampling and conducting subsequent tests.

In sampling a bulk lot of seed the aim was to obtain a sample that truly represented the entire lot. Seed were usually either stored in burlap bags or bins and these containers were used as units for sampling. To obtain a representative working sample for tests involved: (1) Drawing a quantity of seed from each bag approximately proportional to the quantity of seed in all bags and taking this quantity in approximately equal amounts from the top, middle, bottom, and sides of each bag; (2) combining these seeds into a single well-mixed composite sample; and (3) subdividing or drawing at random from the composite to give a working sample of approximately the amount required in the tests.

To illustrate, assume a bulk lot of seed contained in three 100-pound bags and two 50-pound bags. A quart of seed would be drawn from each 100-pound bag, taking randomly one-half pint from near the top, middle, bottom, and sides respectively of each bag.[10] A pint of seed made up of similar drawings from different parts of each container would be drawn from each 50-pound bag. This would give a composite sample amounting to 1 gallon of seed. Random drawings from the composite, totaling about a pint of seed, would furnish a working sample of ample size for these tests.

Samples of seed for testing were drawn from the working sample by pouring the latter in a pile, spreading the seed out in a thin layer, and randomly withdrawing the counted seed. Seed for test samples were withdrawn without discrimination as to size, color, etc., but only plump, apparently sound seed were included, omitting those shriveled, odd-shaped, injured, or otherwise abnormal. Samples of 400 sound seeds for each treatment ordinarily suffice for preliminary tests.

Although any one of several germinating media may be used, tests in sand flats were simple to set up and gave good results. Ordinary packing boxes of any convenient size and shape, filled with clean quartz sand, may be used, but for ease of handling and uniform germination the flat pictured in figure 6 is recommended (*13*).

[10] Samples can be drawn from various parts of a full bag with a grain sampler or similar device. When these are not available, the contents must be poured out to permit sampling. As pointed out on page 27, this must be done eventually and the entire nursery-sowing lot well mixed prior to treatment, hence it is usually advantageous to sample and mix the lot in one operation.

In setting up a test, a flat was filled with clean, moist sand moderately packed to prevent undue settling. After leveling off the sand flush with the top, the surface was marked into 10 evenly spaced drills one-fourth to one-half inch deep. Twenty-five seeds were sown in each drill and covered with dry sand to the drill depth, after which the flat was watered from a watering can fitted with a fine spray, and placed indoors in a well-lighted room where it was sheltered from desiccating wind and sunshine. Rooms with daytime temperatures of 80° F. and more which cool down to 60° or below during the night proved ideal for such tests. The flats were watered frequently—every other day usually being sufficient. Individual drill counts were made daily or periodically of the seedlings reaching the surface. Seedlings were pulled and discarded after counting.

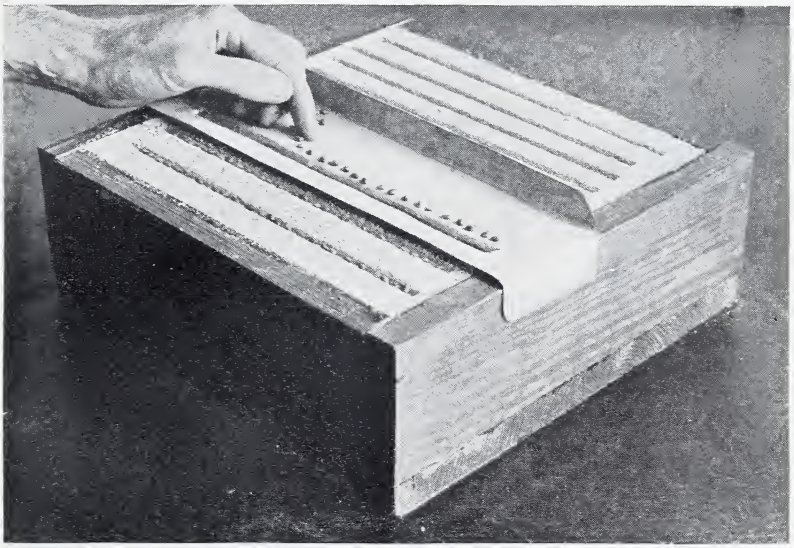

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FIGURE 6.—Sand flat recommended for germination tests. Inside dimensions of flat are 10½ by 10½ by 3½ inches, providing space for 10 drills, each holding 25 seeds. Sides are of 1-inch pine boards, the bottom of similar material or of half-inch pine flooring. Photo shows galvanized iron drill seeder fitted to top of flat with slot placed directly over the third drill from left; this device permits uniform spacing of seed and is operated by distributing seed evenly along the slot, then pushing them through into the drill.

DETERMINING NEED FOR PRETREATMENT

The present studies and those by other investigators indicate that seed of an occasional lot (lot A for example) absorb water readily without treatment of any kind.[11] Such lots possibly possess naturally permeable coats due to environmental or inherent factors or again possibly to the method of collecting and handling—for example, a threshing or cleaning operation which might have partially scarified the seed. Such seed should be satisfactory without pretreatment, and some germination or simple swelling tests should indicate whether pretreatment is needed. These swelling tests could be combined with others made to ascertain the optimum soaking period in acid, but if time permits it would usually be advantageous to conduct them separately and in advance of the latter.

[11] Studies by Chapman (*2*) of seed collected in the Central States indicate that 97 percent of the seed of some lots have permeable coats and imbibe water readily.

Twenty samples of 25 sound seeds each, drawn from the working sample and sown in two sand flats, were found sufficient for these tests. Average germination in 15 days was used as the criterion for judging whether pretreatment was needed. A simpler procedure that indicated approximate germinative capacity consisted of soaking 10 samples of 100 seeds each in water at room temperature for a week or 10 days and counting and averaging the number of seeds that swelled during this period.

Although no hard and fast rules apply to judging the need for pretreatment, it was found to be a safe working rule to exempt from treatment all lots which germinated 60 percent or better in sand flats in 15 days or which gave a comparable value in soaking tests. With lots of this character, it is questionable if acid treatment is justified.

DETERMINING THE OPTIMUM PERIOD OF IMMERSION IN ACID

With the need for treatment satisfactorily established, a knowledge of the optimum period of immersion in the acid was required in advance of actual treating operations. Equipment used in tests for this purpose included: (1) A thermometer; (2) four or more small glass jars or porcelain dishes; (3) a small tea strainer or improvised wire basket; (4) a few ounces of concentrated sulphuric acid; (5) a supply of running water; and (6) eight sand flats.

Five samples of 400 sound seeds each were drawn randomly from the working sample. Choosing a time for treatment when air temperatures were between 60° and 80° F.,[12] four samples were soaked in acid for 30, 60, 90, and 120 minutes, respectively, the other one being left untreated as a check. The treated samples were placed in the jars or dishes, covered with acid, stirred slightly to coat all seed, and allowed to soak for the required periods. At half-hour intervals, a sample was transferred to the strainer, the acid allowed to drain through, momentarily, and the seed brought under a running faucet and rinsed for approximately 3 minutes, after which they were spread out to dry. Air temperatures were observed and recorded: (1) When the acid was poured over all samples, and (2) when the last sample was transferred to the strainer for washing. This was an important observation, since the mean temperature prevailing at this time determined the range in temperatures at which actual treatments were later to be carried on.

After drying but before sowing, the samples were arrayed in order of severity of treatment and the seed scrutinized closely for signs of injury, particular attention being given to ascertaining the longest period of treatment that left 90 percent or more of the seed free of visible evidences of injury. This period was the indicated optimum, although this fact had to be confirmed in subsequent germination tests. Slight injury resulting from treatment was evidenced by small, scattered, often obscure pits which appeared in the seed coat; more severe injury was denoted by patches of definitely corroded cuticle which in some cases became extensive enough to expose the endosperm. Figures 7, 8, and 9 depict samples of untreated, properly treated, and overtreated seed—the last illustrating typical evidences of injury.

[12] If actual treatments are likely to be carried on at temperatures considerably higher than 80°, these tests may be replicated for temperature ranges of 60° to 80°, 81° to 90°, 91° to 100°, etc. Ordinarily tests at temperatures of 60° to 80° are all that are required since locations having temperatures within this range are usually available in basements, well-insulated rooms, etc., even on very hot days. Temperatures below 60° can be easily brought up to the allowable range by artificial heat.

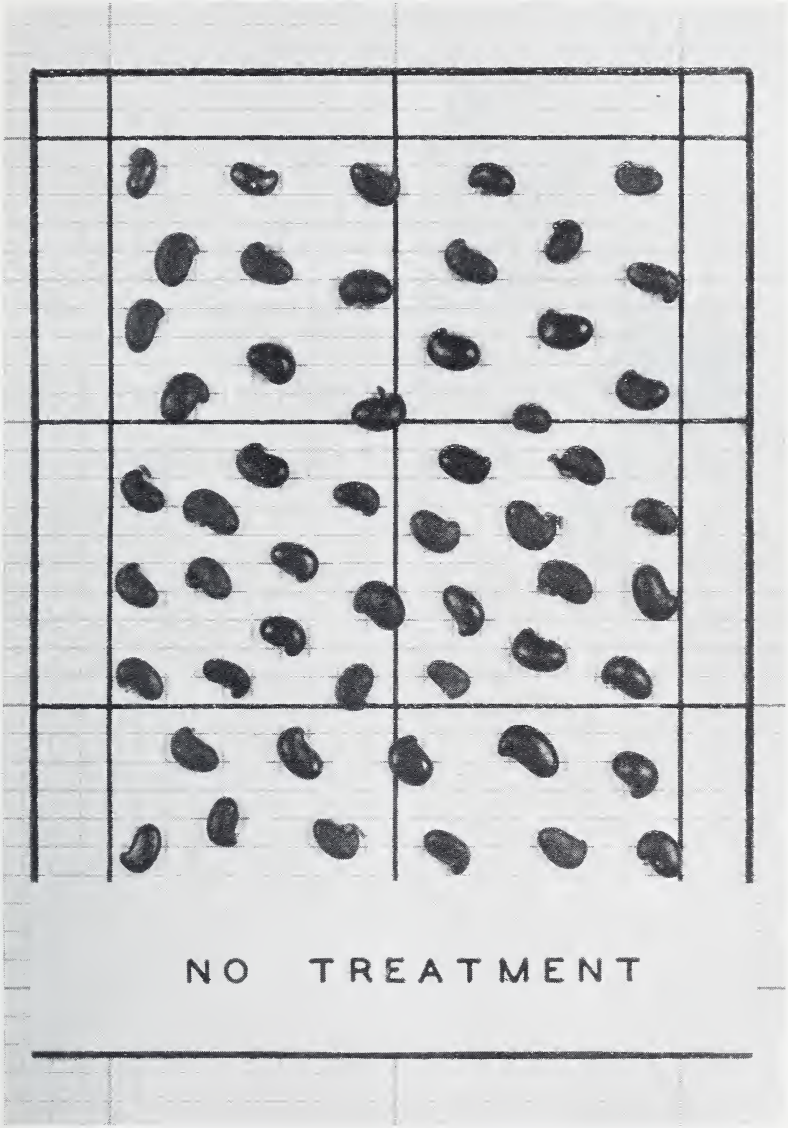

FIGURE 7.—Sample of sound, untreated seed. Approximate enlargement × 2.

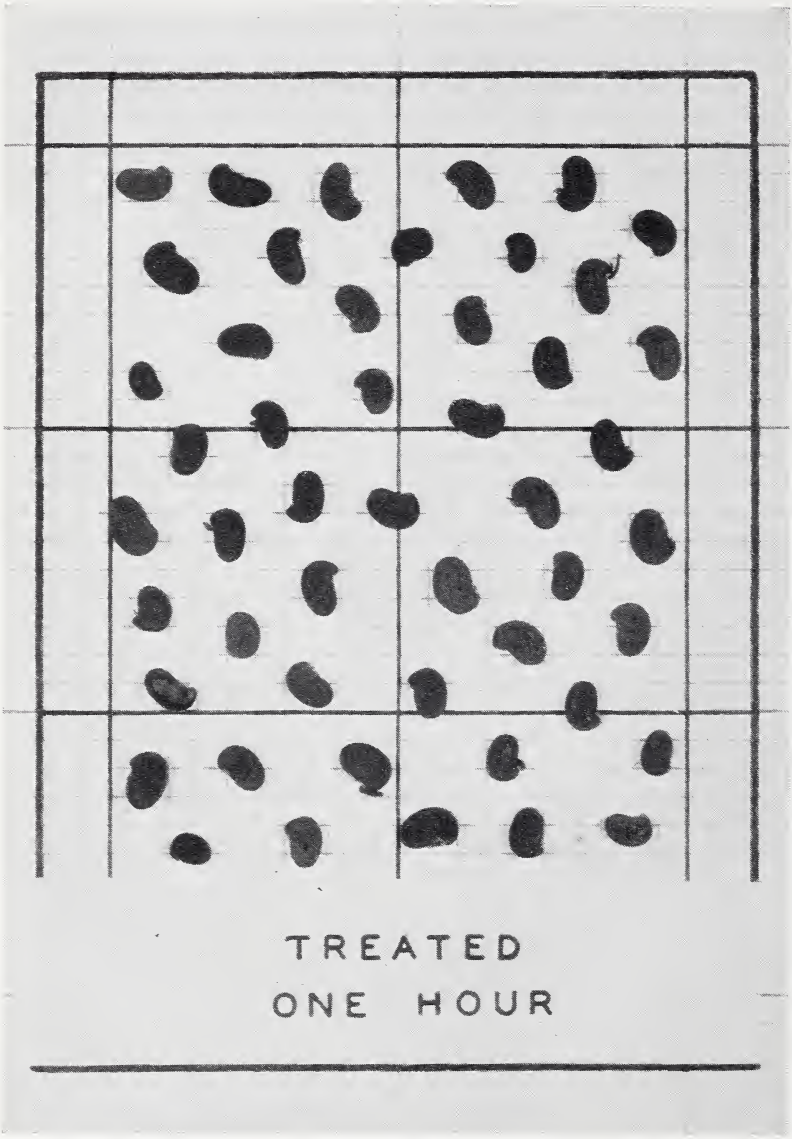

FIGURE 8.—Sample of seed from same lot as pictured in figure 7 given treatment in sulphuric acid for 1 hour. Although lacking in luster, these seed differ little in appearance from those untreated and are practically free of even slight injury to the outer coats.

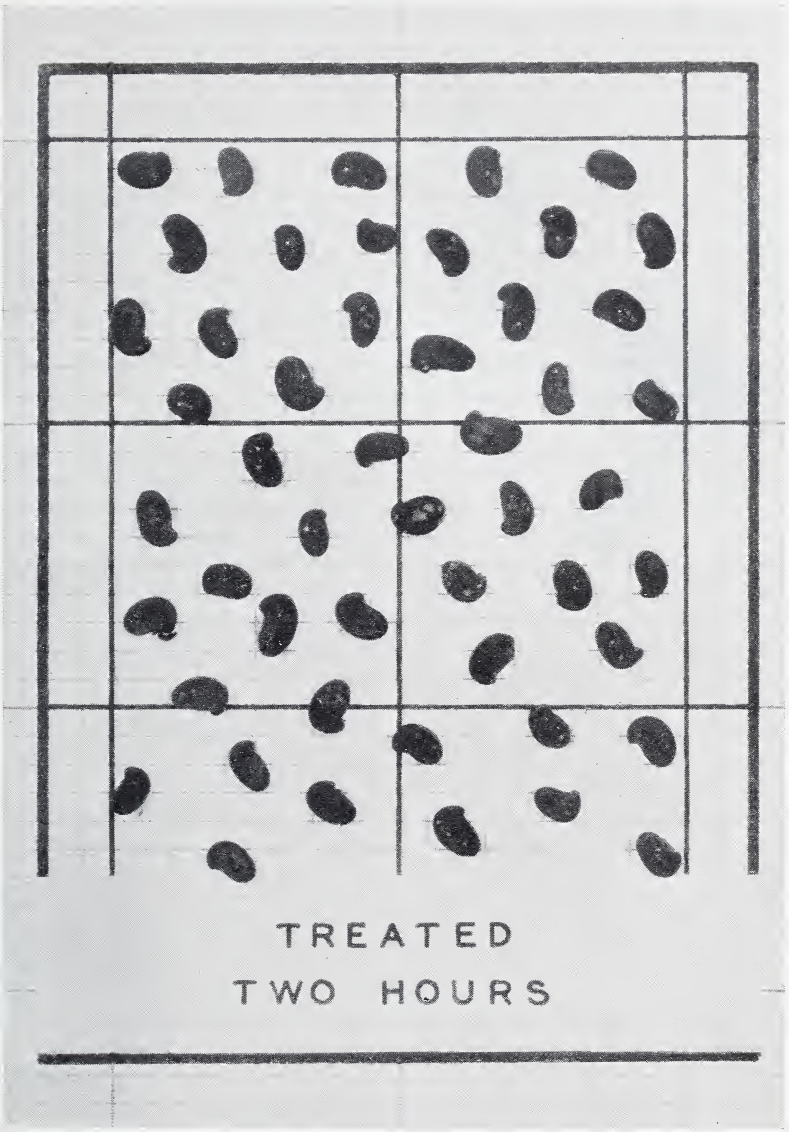

FIGURE 9.—Sample of seed from the same lot as pictured in figures 7 and 8 severely overtreated by steeping in sulphuric acid for 2 hours. Types of injury range from scattered minute pits to extensive corroded areas exposing the endosperm.

After a detailed inspection, each 400-seed sample, including the check, was subdivided into 16 samples of 25 seeds each. A sample of each treatment (check, 30, 60, 90, and 120 minutes) [13] was sown in the first 5 drills of a sand flat, the remaining 5 drills providing 1 complete replication of all treatments and the 8 flats furnishing 16 replications in all. In any one group or block of five drills, treatments were assigned to individual drills in a random or haphazard arrangement rather than in a systematic one. Drills and treatments were suitably identified to facilitate seedling counts and to eliminate errors.

At the end of 15 days the tests were terminated, the total number of seed germinating in each drill ascertained, and average germination for each treatment computed by summing up germination in all 16 drills and dividing the total by 16. These data were modified where necessary according to the extent of observed injuries to seed and used to indicate the proper length of time to expose seed to the acid.

Experience has shown that preliminary germination data, unsupported by other evidence, were not always a reliable index to treatment requirements. Under the favorable germinating conditions in sand-flat tests, partially or even severely damaged seed will sometimes germinate comparatively well, but under field conditions such seed may succumb to drought, disease, and other adversities and germinate more poorly than sound seed receiving a lighter treatment.[14] For this reason, judgment based on the appearance of seed after treatment should always be used in interpreting germination performance and arriving at the proper time to treat. In making this decision, the following rule of thumb was useful: The optimum length of treatment is that producing on an average the greatest germination, provided not more than 10 percent of the seed thus treated show slight pitting or not more than 5 percent show marked corrosion of the outer coats; if these percentages of injury are exceeded the period of treatment should be shortened.

APPLYING THE TREATMENT TO LARGE QUANTITIES OF SEED

With the optimum length of exposure to acid determined, bulk lots of the particular lot of seed in question should be treated at times when temperatures approximate (that is, are ± 5° of the mean of) those in effect when the test treatments were made. Thus, if a mean temperature of 70° F. prevailed during test exposures to acid, seed should be treated at temperatures varying roughly from 65° to 75°. Treatment at temperatures up to 80° would be relatively safe, particularly if the treating time is slightly reduced, but treatment should not be attempted above 80° unless a second series of preliminary tests have been made for a higher range in temperatures. It was found preferable to confine actual treating operations to safe, normal temperatures (60° to 80°) and to deviate very little from allowable limits (10°), in order to employ the acid treatment with certainty and uniformly good results. Ordinarily these temperature

[13] Additional periods of treatment are sometimes desirable, particularly with hard-coated lots requiring a maximum treatment. With most lots, treatment for 120 minutes provides a satisfactory upper limit. If seven treatments are tested, the combination of check, 30, 45, 60, 75, 90, and 120 minutes usually provides an efficient test series. However, the five treatments indicated above will usually suffice.

[14] Seed treated in acid 2 hours (fig. 9) gave germination of 89 percent in sand flats in 13 days, whereas 80 percent of those treated for 1 hour (fig. 8) germinated in the same length of time. In this instance 1-hour treatment was observed to be the longest period leaving more than 90 percent of the seed sound. This observation would fully justify adoption of the shorter period as the optimum. In many such tests there is close agreement between actual germination and observed injuries to seed, particularly where germinating conditions are less favorable than in the tests cited or where germination is observed for a longer period.

restrictions seldom precluded treatment of seed, since locations with desired temperatures can usually be found. During cool weather, room temperatures can be brought up to desired limits by heating, and during hot weather when outdoor temperatures are considerably above 80°, shaded spots, basements, or well-insulated rooms having proper temperatures are usually available for treating operations.

In choosing a site for treatment, the factor of temperature had to be considered in all related details. The acid and such equipment as metal or earthenware containers had to conform with air temperatures and could not be left in the hot sun or near another source of heat just prior to use.

Before treating any portion of a bulk lot of seed from which samples had been drawn for preliminary test, the entire batch was poured out in a single pile on a tarpaulin or tight floor and thoroughly mixed by stirring or shoveling. This was done to render the bulk lot of seed homogeneous and comparable to the composite test sample, thus permitting uniform application of the treatment to all seed comprising the lot.

Only dry seed could be used, and if the seed were damp from being stored in a humid place, they had to be dried thoroughly before acid treatment was attempted.[15]

Ordinary commercial or technical acid of the same specific gravity (1.84) gave almost as good results [16] as chemically pure acid and was much less expensive. It can ordinarily be purchased in lots of several gallons or more for about 4 to 7 cents a pound.

In treating seed, the method found most suitable in large-scale operations as well as for small quantities, was to place the seed in a wire container and lower them into another receptacle containing enough acid to cover them completely. At the end of the treatment the seed container was raised and the acid allowed to drain back into the receptacle, after which the seed were poured into a sieve box and washed in cool water.[17] For treating up to 50 pounds of seed in a single operation, the equipment shown in figures 10 and 11 served admirably. An ordinary steel oil barrel, well cleaned, was used to contain the acid. A wire seed container, circular in cross section, was easily fashioned out of 16-mesh galvanized-wire screen. Several thicknesses of the wire were used and it was reenforced on the outside with 1-inch mesh poultry netting. (Though these materials will in time be corroded by the acid they will withstand many treatments.) The same kinds of wire were used for the bottom of the sieve box in which seed were washed.

Where only a few pounds of seed were to be treated, a simpler method, requiring less equipment but more handling, was to place the seed in a stone jar, porcelain-enameled bucket, or other container of acid-resistant material, and to pour in enough acid to cover them.

[15] Atmospheric humidity has not been known to exert an influence on acid treatment, but theoretically it might be possible for acid exposed to a near-saturated atmosphere to absorb enough moisture during treatment to generate harmful temperatures. Although trial treatments carried on in the humid atmosphere of an ice refrigerator indicated no such effect, it is probably advisable, though not essential, to treat seed during periods of fair weather, and to defer treatments during very humid, rainy spells.

[16] In tests with three lots of seed commercial acid compared in effectiveness with pure acid on an average as follows: Lot C (four tests), 99 percent as effective; lot D (three tests), 88 percent as effective; lot E (two tests), 83 percent as effective. Treatment with commercial acid for slightly longer periods would, of course, offset this advantage.

[17] In treating large quantities of seed requiring 10 to 15 minutes for the acid to drain off, this period should be included in the period of treatment; e. g., 60-minute treatment would consist of immersion in the acid for 45 to 50 minutes plus the period required for draining.

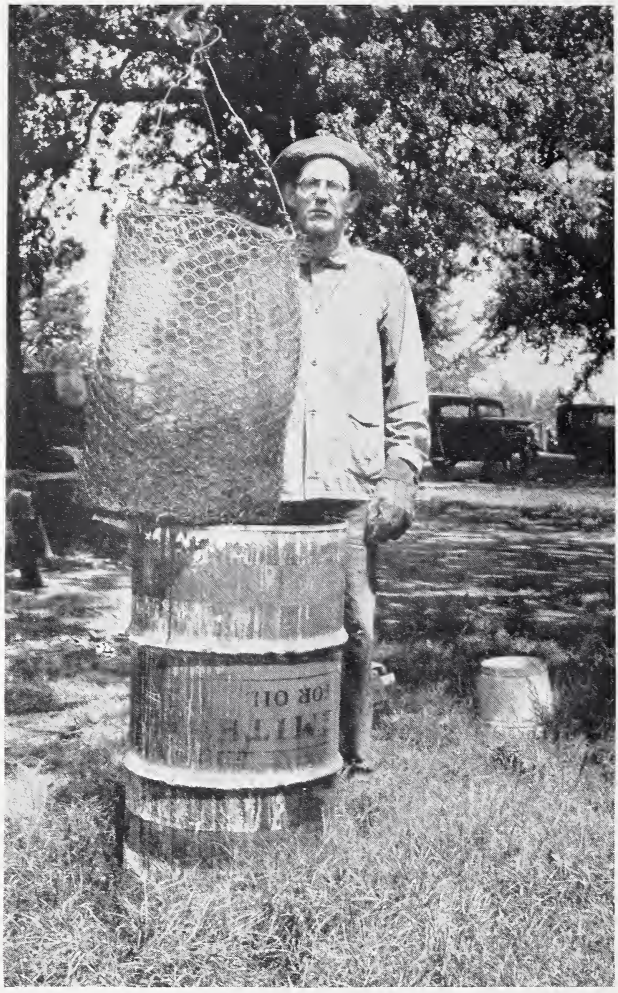

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FIGURE 10.—Equipment used in treating black locust seed with sulphuric acid. The seed, in 50-pound lots, were placed in the wire container and immersed in acid contained in the barrel. After treatment the acid may be drained back into the barrel to be used again.

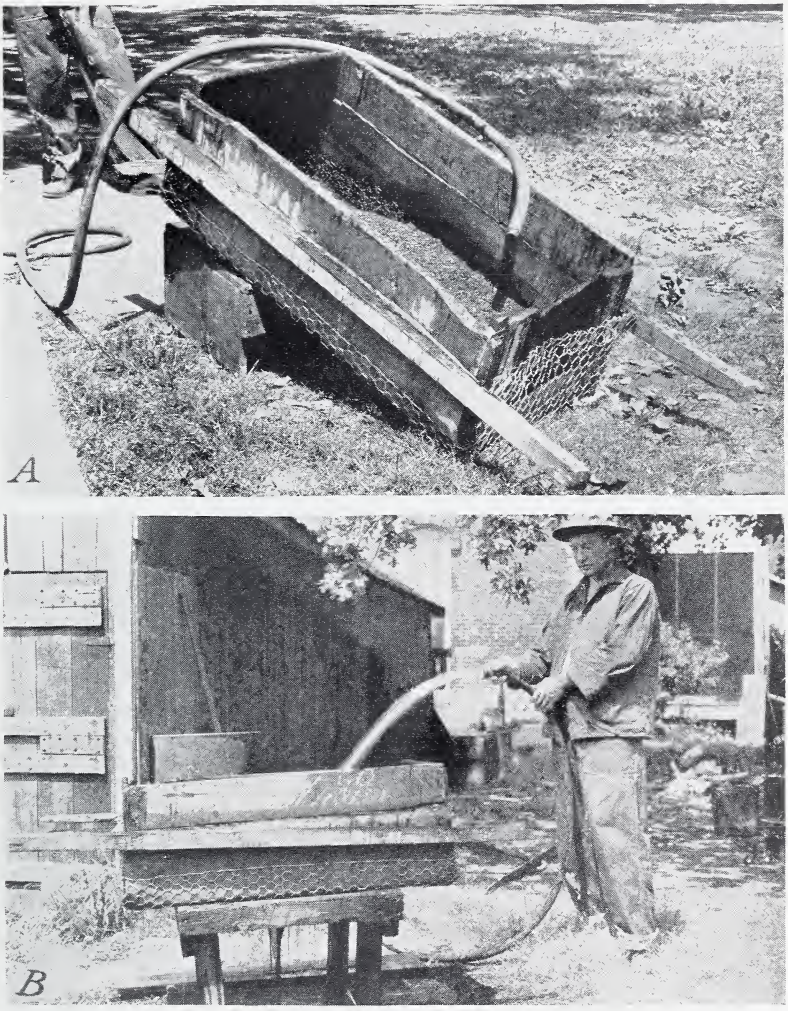

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FIGURE 11.—Black locust seed after steeping in acid is washed thoroughly in cool water: *A*, 50 pounds of treated seed in sieve box ready for washing; *B*, washing the seed; a nozzle adjusted to give a well-distributed spray should preferably be used following a preliminary application of water in large quantities to reduce temperature.

The seed were stirred until all were thoroughly coated with acid. At the end of the treatment they were again stirred slightly—just sufficiently to separate them and not in a violent manner. The seed were then drained in a sieve box, made by replacing the bottom of a wooden packing box with ordinary 16-mesh galvanized-wire screen, or by constructing a basket of several thicknesses of the screen wire. When all of the free acid had drained through, the seed were washed in cool water.

In treating seed by the above methods, 80 to 95 percent of the acid was usually recovered and reused in subsequent treatments. Although it gradually lost its effectiveness [18] and became thick and discolored with use, it was kept at proper strength and consistency by adding fresh acid from time to time. Such additions were also needed to replace unrecovered acid adhering to the seed. The thickened acid rose to the surface on standing overnight and was skimmed off when it became too viscous.

Draining all free acid from the seed before it was dumped into the sieve box to be washed was important also as a precautionary measure to prevent excessive heating of the seed. In washing treated seed, regardless of the quantity, relatively large amounts of water were used at first in order to counteract the heating effect from mixing acid and water. This was especially necessary when as much as 50 pounds of seed was to be washed at one time. Several large buckets of cold water were poured over the seed in rapid succession, followed by routine washing under a stream of cool water. A hose with nozzle adjusted to give a full spray was of great aid, but the water was not applied under strong preassure. The washing period varied with the quantity of seed. Washing for 5 minutes sufficed for small lots of only a few pounds, and for larger lots, up to 50 pounds, about 10 minutes was sufficient; prolonged washing was not only unnecessary but in a few cases was suspected of causing injury.

After seed had been washed, it was immediately spread out in thin layers, preferably in a cool, shaded place, and dried as quickly as possible. Raking at frequent intervals greatly facilitated drying (fig. 12). Piling the seed or placing it in sacks or other containers before it was thoroughly dry was found to be inadvisable.

SUMMARY OF STEPS IN LARGE-SCALE TREATMENT

Summarizing the foregoing application of results the successive steps that resulted in the safe and effective treatment of black locust seed using the sulphuric acid method were:

1. A representative working sample of seed was obtained by random withdrawals from a well-mixed composite sample drawn in proportionate amounts from all bags containing the lot.

2. Whether pretreatment was needed was determined by germinating untreated seed in a sand flat or by soaking samples in water at room temperatures.

3. If less than 60 percent germinated or swelled in 15 days, indicating need of treatment, it was ascertained how long seed were to be soaked in acid for optimum results by germinating in sand flats samples of seed previously steeped in concentrated sulphuric acid for varying periods at air temperatures of 60° to 80° F.

[18] In two tests with seed of lot D, acid which had been used three times was on an average 95 percent as effective as fresh acid; in four later tests, acid used five times previously was on an average 93 percent as effective as fresh acid.

4. The length of treatment that produced on an average the greatest germination in 15 days was adopted as the optimum, provided this treatment was observed to leave 90 percent or more of the seed coats uncorroded by the acid. If more than 10 percent was corroded, a shorter period of treatment was adopted as the optimum—usually the longest treatment observed to leave 90 percent or more of the seed coats entirely sound.

5. Actual treatments based on these tests were confined to times when temperatures approximated (were $\pm 5°$ of the mean of) those prevailing when the test exposures to acid were made. Treatment at temperatures higher than 80° F., although seldom necessary, called for additional tests to determine the optimum treatment for that temperature range.

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FIGURE 12.—Spreading treated seed in a thin layer to facilitate drying.

6. Before treating any portion of a lot of seed, the entire batch was poured together and mixed thoroughly to insure uniform application of the treatment.

7. Using commercial grade acid and equipment described, seed in quantities up to about 50 pounds was steeped in the chemical for the required period, drained well, and washed in cool water for about 10 minutes.

8. After drying well, seed was sacked and stored in a cool, dry place.

CARE REQUIRED IN THE USE OF ACID

Concentrated sulphuric acid is a powerful chemical capable of inflicting serious burns and should always be handled with care. Water reacts violently with the acid, and consequently water should not be added to the chemical or be allowed to splash into the acid container when seed is treated. No danger is involved, however, in washing seed from which the acid has been allowed to drain fully.

In treating operations, care is required chiefly in pouring the chemical from one container to another to prevent it from spattering or spilling down the sides of the container where it may later come in contact with hands or clothing. Care should also be observed in

lowering seed into the acid and in transferring treated seed to the sieve box for washing. If reasonable care is exercised in these details, the treatment introduces no special hazards to workmen, as has been demonstrated in large-scale operations. It is advisable as a special precaution to wear old clothing and gloves, and to keep soap or a weak alkali solution at hand to treat accidental burns.

Bottles or carboys containing the acid should always be well stoppered. The acid should not be kept near inflammables. To prevent deterioration it should be stored in a dark, cool, dry place until ready for use.

COST OF TREATMENT

Costs were variable and, exclusive of the cost of labor and acid, depended on such factors as the quantity of seed treated at one time, the quantity of acid recovered, and the number of times it was reused. Treating an entire lot of seed at a single operation resulted in relatively high costs for acid and equipment. Treatment of small lots in successive operations usually permitted more effective treatment and more complete recovery of the acid. Recovered acid, with small amounts of fresh chemical added, could frequently be used in 10 or more successive operations, thus greatly reducing the cost.

In acid treatments carried on by the Mississippi Forest Service during June 1934 with approximately 2 tons of black locust seed, it was demonstrated that three men could treat about 350 pounds of seed per 8-hour day using a set of equipment such as has been described and pictured. It was found that, in treating 100 pounds of seed, roughly 20 pounds of acid as a maximum was lost in the process. On this basis, with acid costing 7 cents per pound and labor 40 cents per man-hour, the cost of treating the seed was about 4 cents per pound exclusive of a trifling additional cost for equipment.

The same organization treated 4 tons of seed during the 1935 season. Approximately 192 man-hours of labor and 1,750 pounds of commercial acid were required to treat 8,011 pounds of seed. With labor costing 40 cents per man-hour and acid slightly more than 4 cents per pound, costs of the treatment totaled $148.55 or less than 2 cents per pound of seed treated.

In June 1934, the Forest Service treated with acid 200 pounds of black locust seed preparatory to sowing it in a nursery near Holly Springs, Miss. The seed was treated in 8½ pound lots by the second method described above, using 2½-gallon porcelain-enameled buckets and a screen sieve for washing. Four gallons (approximately 61 pounds) of commercial sulphuric acid, purchased for $1 a gallon, was required to treat the entire quantity. The acid was recovered and was reused 12 times. Roughly 25 pounds of acid was used in treating the 200 pounds of seed and the remaining 36 pounds was discarded because of viscosity. Sixteen hours of labor were required to complete the treatment. With labor costing 40 cents per hour and acid costing $4, the total cost of this treatment was $10.40, or 5.2 cents per pound.

In the same locality, in 1935, 300 pounds of seed were treated in a similar operation. Ninety pounds of acid, some of which was saved

for future use, and 24 man-hours of labor were employed. The cost of this treatment amounted to about 4.5 cents per pound of seed.

These costs are probably representative of those likely to be incurred in treating operations using similar types of improvised equipment. Doubtless they can be reduced with increased experience and better organization of the job. More suitable equipment can probably be devised to reduce handling costs and to permit treatment of larger quantities of seed at one time. For example, it might prove feasible to carry out the entire treatment in a single large vat or hopper designed with a special outlet through which acid, rinse water, and seed could be removed in successive operations.

SUMMARY

Experiments conducted by the Southern Forest Experiment Station at Holly Springs, Miss., from 1933 to 1935, showed steeping hard-coated black locust seed in sulphuric acid to be a very effective means of accelerating and increasing germination of the seed. Acid treatment, in addition to being more effective with this type of seed, is more practical than hot-water treatment in large-scale nursery operations, since seed so treated can be more easily sown and can be kept for some time after treatment without loss in viability.

Under field conditions, germination of acid-treated seed compared favorably with that of seed scarified in barrel and disk scarifying machines.

In field tests with four lots of seed, acid-treated seed produced on an average 1.7 to 14.6 times as many seedlings as did untreated seed. In more extensive nursery sowings of the same four lots, the final seedling crop was approximately doubled, ranging from 81 to 114 percent greater from treated seed.

Acid treatment was inexpensive in the examples given, particularly when large quantities of seed were involved, costs ranging from about 2 to 5 cents a pound. With black locust seed costing 25 to 75 cents a pound, a seed treatment capable of doubling seedling yields and costing no more than this would appear to make real savings possible.

Observations and tests show that abnormally high temperatures intensify the action of the acid but that this effect can be compensated for by reducing the period of immersion in the chemical. In practice, however, temperature effects can best be eliminated by restricting experimental trials and subsequent treating operations to periods of normal temperatures, ranging from about 60° to 80° F. Because seed of different lots vary widely with respect to structure and permeability of the outer coats, some lots requiring no treatment at all, preliminary tests for a given temperature range are required to indicate the correct length of exposure to acid. Application of the results are discussed from the standpoint of determining the optimum period of treatment and for treating seed in quantities for nursery sowing.

LITERATURE CITED

(1) Burton, C. L.
1932. variation in characteristics of black locust seeds from two regions. Jour. Forestry 30: 29–33.

(2) Chapman, A. G.
1936. scarification of black locust seed to increase and hasten germination. Jour. Forestry 34: 66–74, illus.

(3) Hurst, W. M., Humphries, W. R., and McKee, R.
1935. barrel and disk seed scarifiers. U. S. Dept. Agr. Circ. 345, 24 pp., illus.

(4) Larsen, J. A.
1925. methods of stimulating germination of western white-pine seed. Jour. Agr. Research 31: 889–899, illus.

(5) Love, H. H., and Leighty, C. E.
1912. germination of seed as affected by sulfuric acid treatment. N. Y. (Cornell) Agr. Expt. Sta. Bull. 312, pp. [293]–336, illus.

(6) Mattoon, W. R.
1930. growing black locust trees. U. S. Dept. Agr. Farmers' Bull. 1628, 14 pp., illus.

(7) Rostrup, O.
1898. report of danish seed control for 1896–97. 37 pp.

(8) Show, S. B.
1917. methods of hastening germination. Jour. Forestry 15: 1003–1006.

(9) Spaeth, J. N.
1932. hastening germination of basswood seeds. Jour. Forestry 30: 925–928.

(10) Todaro, F.
1901. azione dell'acido solforico concentrato su alcuni semi e in particolare sopra i semi duri delle leguminose. Staz. Sper. Agr. Ital. 34: 613–689.

(11) Toumey, J. W., and Durland, W. D.
1923. the effect of soaking certain tree seeds in water at greenhouse temperatures on viability and the time required for germination. Jour. Forestry 21: 369–375.

(12) ——— and Korstian, C. F.
1931. seeding and planting in the practice of forestry; a manual for the guidance of forestry students, foresters, nurserymen, forest owners, and farmers. Ed. 2, rev. and enl., 507 pp., illus. New York and London.

(13) Wakeley, P. C.
1935. artificial reforestation in the southern pine region. U. S. Dept. Agr. Tech. Bull. 492, 115 pp., illus.

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